

Genomics of Cellulosic Ethanol-Producing Bacteria

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Environmental Stress
Pathway Project

VIMSS Virtual Institute for
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University of Missouri-Columbia

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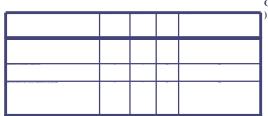
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INTRODUCTION

Recent fluctuations in the global petroleum market have prompted calls for increased research into hability and the very full relative the late are to binderly in particular those presents involving production of binderly in term cellulosic binmass. Here are presented the complete genomic sequences of two strains of ethanol-producing bacteria (Thermonacrobacter psy suchethanolicus 39E and Thermonacrobacter ps. N34). Strain 39E was isolated from the deep subsurface and is predicted to have been geographically isolated from 39E for 259 and isolated from the deep subsurface and is predicted to have been geographically isolated from 39E for 259 as the inversion is particularly interesting as it involves the shuffling and loss of vitamin B1, biosynthesis generally an addition of genomic rearrangements. X514 has also acquired additional Znd-kile heavy metal-translocating P-type ATPase genes through gene duplication and lateral transfer. Metabolic reconstruction reveals further insights into the carbon metabolism and aidac adaptation of the two strains. Both strains are debylogeness serving a the terminal enzyme in the pathway. Slight differences are noted in the carbon metabolism of the two strains, including additional Zndism pathway in 39E, additional pentose metabolism genes in X514 and the lack of a complete methylgloxal shunt in X514. To complement this research, sequencing of the genomes of an additional 20 ethanol-producing Clostridis strains has been research, sequencing of the genomes of an additional 20 ethanol-producing Clostridis strains has been researched as the sequencing of the genomes of an additional 20 ethanol-producing Clostridis strains has been researched sequencing of the genomes of an additional 20 ethanol-producing Clostridis strains has been researched sequencing of the genomes of an additional 20 ethanol-producing Clostridis strains has been researched sequencing of the genomes of an additional 20 ethanol-producing Clostridis strains has been researched as the security of the genomic databa



Metabolic Reconstruction of Inermoanaaerobacter

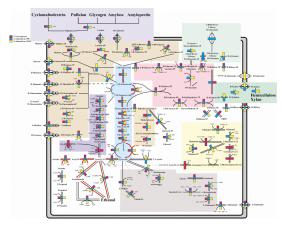
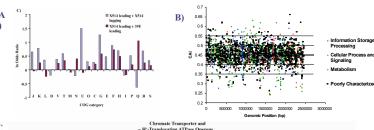


Fig. 1. Metabolic reconstruction of carbon metabolism pathways of sequenced Thermonnerobacter strains. The core pathways of carbon flux from glucose to ethanol are highlighted in red, major pathways are enclosed in colored blocks and enzymes present at each step are color-coded according to the attached legonds. Thermonnaerobacter species employ a novel bifunctional 2° alcohol dehydrogenase as the terminal step in ethanol production as well as a novel amylopullulansae responsible for metabolism of glycogen, amylose and pullulan. Thermonnaerobacter ethanolicus strains are further capable of degrading yglan, but none of the strains studied are capable of cellulose degrading typin, but none of the strains studied are capable of cellulose degrading typin, but none of the strains studied are capable of cellulose degradation.





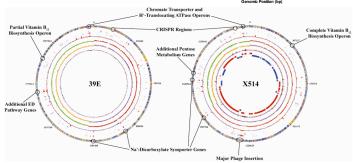


Fig 2. A) Strand bias of X514 and 39E genes based on COG category. X514 encodes more carbohydrate metabolism and transport genes on its leading strand, suggesting these genes may be highly expressed; B) Codon adaptation indices indicate highest (-0.55) and lowest (-0.55) ence expression. Highly expressed genes include those for cellobiose and fructose PTS system components and redox reactions; C) D) From outer ring to inner, COG assignments, laterally transferred genes (by Colombo), GC content, GC-skew, purine bias, SCUO (CodonO), Mauve whole-genome alignment of X514 and 39E (red, conservation in same orientation, blue conservation in opposite orientation)

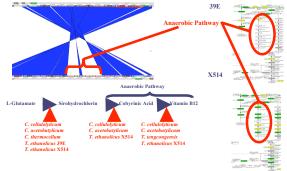


Fig 3. Vitamin B12 biosynthesis pathways of sequenced Clostridia species. B12 biosynthesis is highly variable among Clostridia species, with only a few species encoding complete biosynthesis pathways. The variability may be linked to environment, with strains exiting in low-nutrient environments requiring the full pathway while strains living in higher nutrient environments and/or more complex microbial communities may be able to scavenge exogenous B12 from the environment. Furthermore, in certain strains of Clostridium thermocellum and in some Clostridia cocultures, the presence of exogenous B12 is known to increase the efficiency of ethanol production. The Artemis alignment shows the rearrangement of the B12 biosynthesis pathways in T. pseudoethanolicus strains as the result of a major inversion in the region.

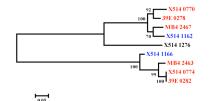


Fig 4. ZntA-like heavy metal translocating P-type ATPases of *Thermoanaerobacter*. Orthologous clusters are labeled red, duplications in X514 are labeled black.

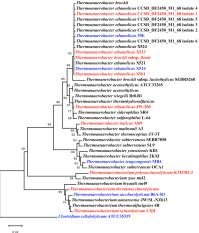


Fig 5. Thermoanaerobacter strains slated for sequencing. Strains in blue represent completed projects, strains in red represent projects initiated or planned by the Zhou laboratory.

ruture Directions

- Evolutionary analyses of *T. pseudoethanolicus* strains (molecular clock assays, positive selection screen, etc.)
- · Physiological analyses of Clostridia single and cocultures

ACKNOWI EDGEMENT

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